Using Ultra-high Carbon Dioxide Levels Enhances Plantlet Growth In Vitro

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Additional index words. CO₂ enrichment, sugar free medium, sucrose, photoautotrophic growth, photomixotrophic growth, heterotrophic growth, lettuce

Summary. A continuous CO₂ flow system was used to study the growth of carrot (Daucus carota L.), citrus (Citrus macrophylla L.), kale (Brassica oleracea L.), lettuce (Lactuca sativa L.), radish (Raphanus sativus L.), and tomato (Lycopersicum esculentum L.) cultures in vitro under photoautotrophic, photomixotrophic, and heterotrophic conditions. Lettuce plantlets were grown on Murashige and Skoog medium with 0%, 0.3%, 1%, and 3% sucrose within flow chambers containing 350, 750, 1500, 3000, 10,000, 30,000, and 50,000 μL·L⁻¹ CO₂. Increasing the levels of CO₂, especially at the ultra-high levels (i.e., ≥3,000 μL·L⁻¹ CO₂), increased fresh weight, shoot length, leaf number, leaf length, leaf width, root number, and root length for plantlets grown regardless of sucrose levels tested compared to plantlets grown at normal atmospheric CO₂ levels, i.e.,
Higher growth rates were obtained when cultures were grown on a medium containing 1% sucrose coupled with an atmosphere enriched with 1000 μL·L⁻¹ CO₂ (photomixotrophic) over that obtained from cultures grown on the same medium without any CO₂ enrichment or on medium devoid of sucrose but enriched with 1000 μL·L⁻¹ CO₂ (Kozai, 1988). Fujiwara et al. (1992) and Fujiwara and Kozai (1995) used 850 to 1000 μL·L⁻¹ CO₂ to double dry weights of potato cultures. At any given CO₂ level tested, dry weight increases were also related to sucrose concentrations suggesting that some combination of sucrose concentration coupled with a certain CO₂ level will give optimum growth (Fujiwara et al., 1992; Fujiwara and Kozai, 1995). Potato shoot cultures cultured on medium devoid of sucrose exhibited up to a 9-fold dry weight increase at CO₂ levels of 10,000 to 50,000 μL·L⁻¹ CO₂ using a passive diffusion flow system (Buddendorf-Joosten and Woltering, 1996; Cournac et al., 1991).

The optimum level of CO₂ for maximum tissue culture growth remains unclear. Based on prior research reports, suggestions as to the proper level of CO₂ to use in vitro varies as much as 10- to 50-fold. This study was conducted to address the influence of various levels of CO₂ on the growth of diverse crop plants cultured in vitro in order to determine optimum levels of CO₂ and sucrose for growth. Also, a low-cost, easily constructed CO₂ passive diffusion continuous flow through system is described that allows for the use of traditional tissue culture vessels to be used in an enriched CO₂ environment. This system could be easily employed by commercial and research laboratories to apply ultra-high (≥3000 μL·L⁻¹) CO₂ levels in vitro.

Materials and methods
Carbon dioxide flow systems.
Carbon dioxide flow-through testing chambers were constructed from a 94.5-L transparent polycarbonate Carb-X tote box and lid (Consolidated Plastics, Twinsburg, Ohio) (45 cm wide × 65 cm long × 37.5 cm in diameter; 94.5-L capacity) (Fig. 1). A silicone tape gasket (112 cm long × 6.3 mm wide × 3.2 mm thick) (Furon, New Haven, Conn.) was attached to the lid (Fig. 1). The box was modified by mounting three polypropylene spigots to allow for the inflow and evacuation of gases. Two 0.45-μm air vents (Gelman Science, Ann Arbor, Mich.) were attached to two of these spigots.

Fig. 1. The plant tissue culture/CO₂ enrichment system. Mixing CO₂ and air generated by air pump is conducted in the flowmeter.
with silicone tubing to 1.6-mm I.D. female barbed fittings (Ark-Plas Products, Flippin, Ark.). The box and lid were clamped with 12 equally spaced stationary binding clips (50 mm long). The CO₂ testing chamber was attached to a water reservoir with silicone rubber tubing. The water reservoir consisted of a 2.25-L polycarbonate bottle containing 1.5-L distilled water. Carbon dioxide was provided by a gas cylinder (National Welding Supply Company, Inc., Bloomington, Ill.) rated 99.8% pure and was mixed with room air flow produced by an aquarium pump (Whisper 1000, Carolina Biological Supply Co., Burlington, N.C.) with a flowmeter (Cole Parmer Instrument Co., Niles, Ill.) to provide 350, 750, 1500, 3000, 10,000, 30,000, and 50,000 μL·L⁻¹. Carbon dioxide ranges ≥10,000 μL·L⁻¹ were adjusted using an infrared gas analyzer (model 3000 LIRA; Mine Safety Appliances Co., Pittsburgh) and CO₂ ranges ≤3,000 μL·L⁻¹ were adjusted with the aid of a CO₂/H₂O infrared gas analyzer (LI-6262; LI-COR, Lincoln, Nebr.). The CO₂ and air streams were added at about 1500 mL·min⁻¹ for a 16-h photoperiod. Control cultures were given a stream of room air generated by the aquarium pump and hydrated with a water reservoir. In flow experiments, air flow rates were adjusted with gang valves and flowmeters to 250, 500, 1000, 1500, and 2000 mL·min⁻¹. Flow experiments were conducted using 17.6-L transparent polycarbonate boxes (Consolidated Plastics, Twinsburg, Ohio) (32.5 cm long × 26.3 cm wide × 30 cm high; 17.6-L capacity) equipped with a silicone gaskets, vents, and water reservoirs as described above. In some cases, this system was modified by including a 905 mL·min⁻¹ VAC fan (5.7 × 3.8 cm).

**Diffusion Tests.** Diffusion tests were conducted on the following tissue culture vessels: culture tubes (25 mm in diameter × 150 mm high or 38 mm in diameter × 200 mm high) capped with a polypropylene natural closure (Corning, Boston); Magenta GA-7 container (77 mm wide × 77 mm long × 97 mm high) capped with polypropylene closure (Sigma Chemical Co., St. Louis); and a Phytaco (116 mm in diameter × 108 mm high) (Sigma Chemical Co.) capped with a polypropylene cover. An O₂ sensor (Figaro USA, Wilmette, Ill.) was placed inside the culture vessel to be tested either by passing the wires from the sensor through an opening obtained by drilling a hole in the closure or by inserting a tube into the vessel and sealing the wires with silicone sealer. For the 25 mm × 150-mm tube a sidearm was added to the tube for the O₂ sensor. A CO₂ electrode (Diamond General, Ann Arbor, Mich.) was used to measure CO₂ and was inserted into the vessel through a hole drilled in the closure. Nitrogen/CO₂ (80%/20%) gas was passed into the vessel through a tube either inserted into the closure or attached to the vessel. Once zero O₂ was recorded within the vessel, gas input was terminated and O₂ and CO₂ levels were recorded until normal atmospheric O₂ (i.e., 21%) or CO₂ level (i.e., 0.03%) was obtained. The recorded sensor outputs were converted to the estimated number of air exchanges per hour (N) using the following formula: 

\[ N = \left( \frac{1}{t_2 - t_1} \right) \ln \left( \frac{C_1 - C_{out}}{C_2 - C_{out}} \right) \]

where \( t_1 = \) time when vessel concentration = \( C_1 \); \( t_2 = \) time when vessel concentration = \( C_2 \); and \( C_{out} = \) concentrations outside vessel (Fujitawara and Kozai, 1995). Air exchange rates were also conducted by inserting a CO₂ probe into a capped 25 mm × 150-mm culture tube within a flow-through system with and without a fan using 30,000 μL·L⁻¹ CO₂.

**Medium and Plant Culture.** The basal medium (BM) consisted of Murashige and Skoog (MS) salts plus (per liter): 0.5 mg thiamine HCl, 100 mg myo-inositol, and 10 g agar (Difco Laboratories, Detroit). BM with 0%, 0.5%, 1%, 2%, and 3% sucrose and 3% 4% sucrose was prepared. The pH was adjusted to 5.7 ± 0.1 with 0.1 N HCl or NaOH before the addition of agar, then melted and dispensed in 25-mL aliquots into 25 mm × 150-mm borosilicate glass culture tubes and capped with translucent polystyrene closures (Sigma Chemical Co., St. Louis). The medium was autoclaved for 15 min at 1.05 kg-cm⁻² at 121 °C. Agar medium was then slanted at a 45° angle while cooling. Seeds of carrot (Daucus carota L. 'Danver's Half Long'), kale (Brassica oleracea L. unknown cultivar), lettuce (Lactuca sativa L. 'Grand Rapids'), radish (Raphanus sativus L. 'Scarlet Globe'), and tomato (Lycopersicum esculentum L. 'Cherry Red') were surface sterilized in a 2.6% sodium hypochlorite solution (containing 2 drops of Tween-20 emulsifier per 100 mL solution) for 20 min and placed on the surface of BM. Two seeds were cultured per vessel. Stock shoots of citrus (Citrus macrophylla L. unknown cultivar) were maintained as proliferating axillary buds on BM. A single 2-cm-long shoot was cultured per vessel.

**Plant Tissue Culture Experiments.** Lettuce seeds were planted in BM containing 0%, 0.3%, 1%, 2%, or 5% sucrose and grown under 350, 750, 1500, 3000, 10,000, 30,000, and 50,000 μL·L⁻¹ CO₂ in 94-L transparent containers. Carrot, citrus, kale, radish, and tomato were similarly grown at the same concentration but on BM with and without 3% sucrose. The influence of the CO₂ flow rates on lettuce seedling growth was tested by growing lettuce seeds on BM with sucrose under 10,000 μL·L⁻¹ CO₂ using 250, 500, 1500, and 2000 mL·min⁻¹ within 17.6-L transparent containers. Cultures were grown in a culture room maintained at 25 ± 1 °C and using a photoperiod of 16 h light/8 h dark. Light was supplied by a combination of cool-white fluorescent tubes, metal halide lamps, and incandescent lamps.

**Fig. 2. Growth responses of lettuce plantlets to various concentrations of CO₂ and sucrose.** Two seeds were cultured per tube. Data were averaged for 10 replications/treatment. Experiments were repeated three times and a single representation is presented. Mean separation by Student-Newman-Keuls multiple range test (P < 0.1). Columns in the same sucrose concentration with the same letter were not significantly different.

**Table 1. Oxygen and carbon dioxide exchange rates (N) for various tissue culture vessels.**

<table>
<thead>
<tr>
<th>Vessel type</th>
<th>Capacity (mm²)</th>
<th>O₂ (h⁻¹)</th>
<th>CO₂ (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture tube (25 × 150 mm)</td>
<td>55</td>
<td>2.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Culture tube (38 × 200 mm)</td>
<td>175</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Magenta GA-7 container</td>
<td>350</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Phytaco container</td>
<td>470</td>
<td>0.8</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Note: These values are theoretical and were calculated based on the diffusion model and the experimental conditions.
Fig. 3. Growth responses of lettuce plantlets given various CO₂ levels in vitro after 8 weeks in culture. (A) Plantlets grown on medium without sucrose treated with 350 and 10,000 μL-L⁻¹ CO₂. Note the difference in the size of plantlets depending on whether CO₂ was enhanced or not. (B) Plantlets treated with 10,000 μL-L⁻¹ CO₂ cultured in various concentrations of sucrose. Note that the sucrose levels do not alter growth responses at this level of CO₂ tested. Scale bar = 16 mm.

at a total photosynthetic photon flux density (PPFD) of 260 μmol·m⁻²·s⁻¹ at the vessel periphery.

Ten or twenty replicates were planted originally, and experiments were repeated at least twice. After 8 weeks of incubation, data on culture dry weight, fresh weight, shoot height, leaf number, leaf length, leaf width, root number, and root length were recorded and analyzed with Student-Newman-Keuls multiple range test as appropriate.

Results and discussion

Diffusion results in terms of the O₂ and CO₂ exchange rates (N) for the various culture vessels are given in Table 1. For the same vessel type, O₂ and CO₂ exchange rates were similar. The 25 × 150-mm tube gave the best air exchange rates followed by the 38 × 200-mm tube, the Sigma vessel, and the Magenta vessel, respectively. The choice to use 25 × 150-mm test tubes in this study was made based on this test because faster air exchanges could be obtained with these vessels and closures compared to any other commonly used tissue culture vessels. Using a flow-through system with a fan allowed for faster air exchange than without a fan, 2.07 and 1.39 h⁻¹, respectively. Without a fan, air exchange was only about 70% as fast in the flow-through system than with a fan. In other words, the time to achieve a given concentration in 60 min using a system with a fan will require 85 min in a system without a fan. This increased time is negligible over a daily 16-h period of CO₂ application, and a fan was not used in subsequent tests.

Levels of CO₂, especially above the 1500 μL-L⁻¹ CO₂ level, resulted in significant lettuce plantlet growth increases in BM containing 0% to 3% sucrose (Fig. 2). However, 5% sucrose was inhibitory to plantlet growth for all CO₂ concentrations tested compared to lower sucrose concentrations tested and will not be considered in the following discussion hereafter (data not shown). Invariably, administration of air containing 350 μL-L⁻¹ CO₂ gave the least growth response from cultured lettuce seedlings, followed by 750 μL-L⁻¹ CO₂, regardless of the sucrose levels tested. Highest growth responses were obtained using 10,000, 30,000, and 50,000 μL-L⁻¹ CO₂ compared to 350 μL-L⁻¹ CO₂ (Figs. 2 and 3). The greatest fresh weights obtained were from lettuce grown in 30,000 μL-L⁻¹ CO₂, regardless of the levels of sucrose used. For example, lettuce plantlets grown in 0% and 3% sucrose under 30,000 μL-L⁻¹ CO₂ exhibited 13.8- and 7.2-fold increases, respectively, in growth over cultures grown in air only on the same media. The high photomixotrophic condition (3% sucrose with high CO₂ levels) usually gave similar results compared to the autotrophic condition (0% sucrose with high CO₂ levels) or the low photomixotrophic condition (0.3% or 1% sucrose with high CO₂ levels). All of these treatments were superior to the heterotrophic condition (0.3% to 3% sucrose without any CO₂ enrichment). Low sucrose levels coupled with enriched CO₂ environments have been reported to give higher dry weights from carnation, orchids, and potato cultures than those obtained from cultures grown on no or higher sucrose levels devoid of CO₂ enrichment (Fujiwara et al., 1992; Kozai, 1988).

With lettuce this pattern did not occur; however, considerably higher levels of CO₂ were used in the present study (Figs. 2 and 3). We found that greater increases in growth (i.e., fresh weight) occurred from lettuce cultures with 30,000 μL-L⁻¹ CO₂-treated cultures at the 0% sucrose levels than with any other sucrose levels used (Fig. 2). No notable beneficial effect occurred using BM containing 0.3% or 1% sucrose compared to using BM containing 0% or 3% sucrose for any of the growth responses recorded. Shoot lengths of lettuce plantlets were similar when cultured under 3000 to 50,000 μL-L⁻¹ CO₂, regardless of the sucrose levels used. The highest number of leaves per culture, leaf lengths, and leaf widths occurred using the 30,000 to 50,000 μL-L⁻¹ CO₂ levels compared to the lower CO₂ levels, regardless of the sucrose levels used (Fig. 2). Similarly, Buddendorf-Joosten and Woltering (1996) found that leaf number and leaf area significantly increased growing potato shoots at 50,000 μL-L⁻¹ CO₂ compared to the cultures grown in air only. Cacao shoot cultures cultured on 20,000 μL-L⁻¹ CO₂ exhibited enhanced leaf area production and shoot elongation compared to those cultured at 800 μL-L⁻¹ CO₂ (Figueira et al., 1991).

Carbon dioxide enrichment in vitro has been associated with growth responses such as increases in dry weight (Cournac et al., 1991; Fujiwara et al., 1992; Kozai et al., 1987), plant height (Cournac et al., 1991; Figueira et al., 1991), fresh weight (Buddendorf-Joosten and Woltering, 1996), or leaf area (Buddendorf-Joosten and Woltering, 1996; Figueira et al., 1991). Lettuce plantlet root responses, i.e., root number and root lengths, were significantly improved when cultured under ultra-high levels of CO₂ (≥3,000 μL-L⁻¹) for all sucrose levels tested (Figs. 2 and 3). The beneficial effect of CO₂ on rooting in vitro has not been mentioned in previous reports.

Based on the previous experiments, ultra-high levels of CO₂ (≥3,000 μL-L⁻¹) were found to be beneficial to lettuce plantlet growth. Several differ-
different species were cultured on the same elevated levels of CO$_2$ on BM with or without sucrose, and high concentrations of CO$_2$ aided in the growth of all species studied (Fig. 4). However, an optimum concentration of CO$_2$ appeared to exist for each species where above or below this concentration less growth occurs (i.e., fresh weight). For example, citrus shoots grown in 0% sucrose exhibit maximum growth at the 10,000 μL·L⁻¹ CO$_2$ level while kale seedlings grown in 0% sucrose exhibit maximum growth at the 30,000 μL·L⁻¹ CO$_2$ level. Also, it was observed that maximum growth increases (i.e., fold increases) were not always the same when grown in 0% or 3% sucrose. For example, radish and tomato exhibited higher growth rates on BM with sucrose than on BM devoid of sucrose, regardless of the CO$_2$ concentration, while kale and citrus exhibited higher growth rates on medium devoid of sucrose. Apparently, a species-related response to CO$_2$ levels coupled with the sucrose concentration occurred to modify the growth responses observed. Except for tomato, greater fresh weight increases occurred on BM without sucrose at a certain optimum level of CO$_2$. This observation agrees with that observed by Kozai (1988) with other species. The optimum CO$_2$ level differed somewhat among species and media sucrose concentration but, generally, 3000 to 30,000 μL·L⁻¹ CO$_2$ levels were found to give the largest fresh weight increases (Fig. 4). Carrot exhibited maximum fresh weight increase of 9.5-fold on BM without sucrose with 30,000 μL·L⁻¹ CO$_2$, but on BM with sucrose, a maximum fresh weight of only 1.7-fold occurred with 10,000 μL·L⁻¹ CO$_2$. The effect of ultra-high levels of CO$_2$ on the different species (e.g., carrot) was visibly obvious when compared to growth obtained at 850 μL·L⁻¹ CO$_2$ (Fig. 5). Similarly, kale plantlets exhibited their maximum fresh weight response, a 6.5-fold increase, on BM without sucrose with 30,000 μL·L⁻¹ CO$_2$; on BM with sucrose, only a 1.7-fold increase in fresh weight occurred on 3000 μL·L⁻¹ CO$_2$. Citrus shoots exhibited a maximum fresh weight increase of 4.7-fold on BM without sucrose with 10,000 μL·L⁻¹ CO$_2$, but on BM with sucrose only a maximum of 1.3-fold increase with 10,000 μL·L⁻¹ CO$_2$. Radish plantlets exhibited a maximum fresh weight increase of 6.3-fold on BM without sucrose with 10,000 μL·L⁻¹ CO$_2$, and only a 3.3-fold increase on BM with sucrose with 30,000 μL·L⁻¹ CO$_2$. Tomato plantlets exhibited a maximum fresh weight increase of 0.8-fold on BM without sucrose with 3000 μL·L⁻¹ CO$_2$ and a 1.2-fold on BM with sucrose with 10,000 μL·L⁻¹ CO$_2$. Associated with increasing fresh weights are

![Fig. 4. Growth responses of several plants to various levels of CO$_2$ in vitro after 8 weeks in culture. Two seeds each of carrot, kale, radish, and tomato were cultured per tube. For Citrus one 2-cm-long shoot culture was planted. Data were averaged for 10 replications/treatment. Experiments were repeated three times and single representation is presented. Mean separation by Student-Newman-Keuls multiple range test (P < 0.1). Columns for the same sucrose concentration with the same letter were not significantly different.](image-url)
also increases in shoot length, leaf number, leaf length, leaf width, root number, and root length (data not shown).

Differences in growth responses of various species cultured under various levels of CO2 have been reported previously (Fujiwara et al., 1988; 1992; Jeong et al., 1993; Kozai et al., 1987). Our results indicate that certain levels of CO2 cause optimum growth responses depending on the species and medium sucrose concentration. It can be concluded from these experiments that CO2 administered at a wide range of concentrations aids in the growth of sterile seedlings; however, ultra-high levels of CO2 (≥3,000 μL·L−1) are particularly advantageous in the in vitro growth of most plantlets regardless of the inclusion or omission of carbohydrates from the BM. These results contrast to those reported from previous greenhouse studies that suggest 1000 μL·L−1 CO2 is optimum for most plants (Enoch and Kimball, 1986). The use of CO2 >1000 μL·L−1 CO2 level is considered unnecessary and is often detrimental to the growth of plants (Enoch and Kimball, 1986). Similarly, Kozai et al. (1987) found that 1000 μL·L−1 CO2 levels gave high growth rates with a number of different plants (e.g., carnation, orchid, and potato) in vitro. However, other researchers have used higher levels of CO2 (10,000 to 50,000 μL·L−1 CO2) to enhance plant growth (Buddendorff-Joosten and Woltering, 1996; Cournac et al., 1991; Figueira et al., 1991; Treat et al., 1989). In our study, the merits of using high CO2 (750 and 1500 μL·L−1) and ultra-high CO2 (≥3000 μL·L−1) concentrations were evaluated on six species. Both ranges of CO2 enhanced the growth of plantlets in vitro. However, the ultra-high concentrations of CO2 consistently gave better results and will merit serious consideration in further CO2 testing research with other plants. In addition, the optimum level of CO2 must be determined empirically for each plant.

The CO2 flow rate when varied from 250 to 2000 mL·min−1 was not critical to the growth of lettuce plantlets (Table 2). There was no effect of CO2 flow rate on the growth of lettuce seedlings over the flow rates tested. Flow rates administered to cultures have varied in the literature greatly ranging from as low as 10 mL·min−1 (Prusky et al., 1996) to as high as 5000 mL·min−1 (Cournac et al., 1991). The flow rate becomes critical depending on the size of the chamber. In this study, the 250–mL·min−1 rate displaces 17.6 L of chamber air in 70 min. This corresponds to other experiments where 1500 mL·min−1 was applied to the larger 94.5-L chambers, which took 62.6 min to displace this volume. Significant modifications of the gaseous composition occurs during the growth of cultures within vessels (Kozai, 1988). Volatile products such as ethylene and other hydrocarbons may accumulate and inhibit plant growth and development. In the flow-through system presented in this study, it is likely photosynthesis is enhanced by elevated CO2 levels coupled with the added benefit of the removal of volatiles (Buddendorff-Joosten and Woltering, 1994).

**Remarks**

Photoautotrophic and photomixotrophic conditions were achieved in a flow-through system and a wide range of CO2 concentrations was tested on several plant species within traditional tissue culture vessels. It should be pointed out that use of special filters or vessels was not necessary to use a enriched CO2 environment, provided the culture vessel and closure provides for sufficient ventilation. The typical tissue culture tubes and closures satisfied this requirement. Light intensity is critical to obtain optimum benefit from high CO2 levels. Our experiments were conducted under 260 μmol·m−2·s−1 while the typical tissue culture room uses 40 to 100 μmol·m−2·s−1. Cournac et al. (1991) found that 1000 μmol·m−2·s−1 caused potato cultures supplemented with 10,000 μL·L−1 CO2 to grow better than cultures under 230 μmol·m−2·s−1 at the same CO2 levels. Similarly, we found higher growth rates for our cultures when given 260 instead of 120 μmol·m−2·s−1 (data not shown). High light intensity coupled with high and ultra-high CO2 levels used in our experiments were responsible for the autotrophic growth of cultures on BM without sucrose. We conclude that a wide range of CO2 enhances plantlet growth in vitro; however, ultra-high levels of CO2 (≥3,000 μL·L−1) consistently gave better results than high CO2 levels (750 to 1500 μL·L−1). Ultra-high levels of CO2 may completely eliminate the requirement of an external carbohydrate source for culture survival and optimum growth. Frequently, culture growth on BM without sucrose (photoautotrophic

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**Table 2.** Influence of various flow rates 10,000 μL·L−1 CO2 on the growth of lettuce plantlets in vitro. Cultures were grown in 17.6-L transparent containers. Carbon dioxide was administered for 16 h·d−1 for 8 weeks.

<table>
<thead>
<tr>
<th>Flow (mL·min−1)</th>
<th>Leaves per culture</th>
<th>Shoot length (mm)</th>
<th>Culture wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>11.0 ± 0.6 a</td>
<td>95.0 ± 15.0 a</td>
<td>5.8 ± 1.0 a</td>
</tr>
<tr>
<td>500</td>
<td>11.8 ± 0.3 a</td>
<td>95.0 ± 15.0 a</td>
<td>6.6 ± 0.1 a</td>
</tr>
<tr>
<td>1500</td>
<td>11.4 ± 0.4 a</td>
<td>98.3 ± 11.7 a</td>
<td>6.6 ± 1.6 a</td>
</tr>
<tr>
<td>2000</td>
<td>12.5 ± 0.9 a</td>
<td>90.0 ± 15.3 a</td>
<td>7.2 ± 0.8 a</td>
</tr>
</tbody>
</table>

*Treatment averages and ± presented. Mean separation within growth response columns by Student-Neuman-Keuls multiple range test (P≤0.1).*
condition) exceeded that occurring in BM containing sucrose (photomixotrophic condition) for several plants (e.g., carrot and lettuce) if given an adequate CO₂ level (Fig. 4). We conducted our studies with different species and obtained significant growth increases using ultra-high levels of CO₂. This work suggests that other species should now be tested using the inexpensive CO₂ flow-through system presented herein with similar CO₂ levels.

**Literature Cited**


Time course of CO₂ exchange of potato cultures in vitro with different sucrose concentrations in the culture medium. J. Agr. Met. 48:49-56.


